

pBOSS1

(8398 bp)

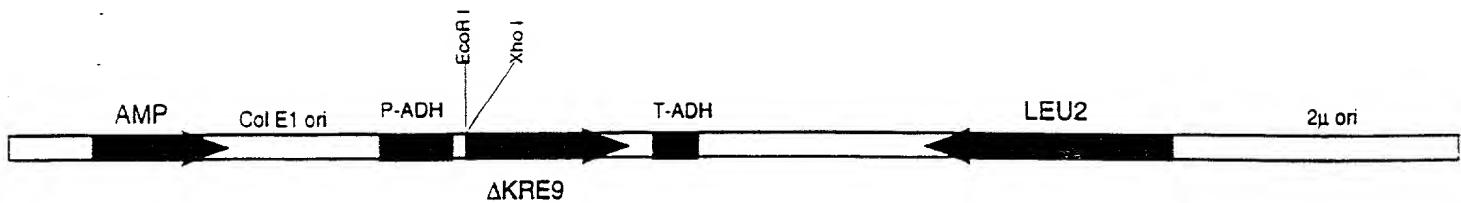


FIG. 1A

SIGNAL PEPTIDE SELECTION IN pBOSSI

- Step 1 Transform library into Δ KRE9 yeast strain
Titer on galactose
- Step 2 Plate transformants on glucose
- Step 3 Harvest colonies at day 2-4, rescue plasmid DNA in batch
- Step 4 Transform E.coli DH10B, sequence ends of rescued plasmids

FIG. 1B

GGGGACCGTGTGCCCCAAGCC	AGCCCCCATTGGAACTCAGCGAGTAGGG	GGCTCTGGGAAGTGG	79
CAGGGGGCGCAGCAGCTGCTGCCACTCCAGGTGCTGAAGAGGATCTCGGAGCCGCTCTGCC	CCCAGGG	158	
CGCTGGATGACTGGCACCA	GCGCTCCACCTGTGTTGGTGTGAGACTTGGCTGGAGTCCCACGTGGCTGTGG	237	
AGTCAGTGTGATT	CATGATTGAGGAAACCGCTCCATCCTCTCTCCTGGCAC	TTCCACACATGAGGAAGAA	316
M K G T C V I		7	
GAGCTTCTGTTAGAAGACACGTGCCAGAGTCAGAGGCCCTGCCACC	ATG AAG GGA ACC TGT GTT ATA	388	
A W L F S S L G L W R L A H P E A Q G T		27	
GCA TGG CTG TTC TCA AGC CTG GGG CTG TGG AGA CTC GCC CAC CCA GAG GCC CAG GGT ACG		448	
T Q C Q R T L E V N I V S P S S K A T F		47	
ACT CAG TGC CAG AGA ACA CTC GAG GTG AAT ATT GTT TCC CCC AGC TCC AAG GCA ACA TTC		508	
S P S (SEQ ID NO:2)		50	
AGT CCA AGT (SEQ ID NO:1)		517	

FIG. 2

TTCTTCCTAGTTCTTTTCCGCACAATA...TCAAGTTATACCAAGCATAACAATCAACTCCC...TTGGGATCCGAATT 79

CGGCACGAGCGGCACCGAGTTGTGCTTCGGAGACCGTAAGGATATTGATGACC	ATG	AGA	TCC	CTG	CTC	AGA	M	R	S	L	L	R	6							
													149							
T	P	F	L	C	G	L	L	W	A	F	C	A	P	G	A	R	A	E	E	26
ACC	CCC	TTC	CTG	TGT	GGC	CTG	CTC	TGG	GCC	TTT	TGT	GCC	CCA	GGC	GCC	AGG	GCT	GAG	GAG	209
P	A	A	S	F	S	Q	P	G	S	M	G	L	D	K	N-	T	V	H	D	46
CCT	GCA	GCC	AGC	TTC	TCC	CAA	CCC	GGC	AGC	ATG	GGC	CTG	GAT	AAG	AAC	ACA	GTG	CAC	GAC	269
Q	E	H	I	M	E	H	L	E	G	V	I	N	K	P	E	A	E	M	S	66
CAA	GAG	CAT	ATC	ATG	GAG	CAT	CTA	GAA	GGT	GTC	ATC	AAC	AAA	CCA	GAG	GCG	GAG	ATG	TCG	329
P	Q	E	L	Q	L	H	Y	F	K	M	H	D	Y	D	G	N	N	L	L	86
CCA	CAA	GAA	TTG	CAG	CTC	CAT	TAC	TTC	AAA	ATG	CAT	GAT	TAT	GAT	GGC	AAT	AAT	TTG	CTT	389
D	G	L	E	L	S	T	A	I	T	H	V	H	K	E	E	G	S	E	Q	106
GAT	GGC	TTA	GAA	CTC	TCC	ACA	GCC	ATC	ACT	CAT	GTC	CAT	AAG	GAG	GAA	GGG	AGT	GAA	CAG	449
A	P	L	E	V	N	I	V	S	P	S	S	K	A	T	F	S	P	S	(SEQ ID NO: 1)	125
CGCA	CCA	CTC	GAG	GTG	AAT	ATT	GTT	TCC	CCC	AGC	TCC	AAG	GCA	ACA	TTC	AGT	CCA	AGT		506
																				(SEQ ID NO: 3)

FIG. 3

Comparison of novel protein sequence emxosb4a11
to murine semaphorin F (Genbank Accession number X97817)

Identities = 22/32 (68%), Similarities = 26/32 (81%)

emxosb4a11:	1 <u>MKGTCVIAWLFS</u> SLGLWRLAHPEAQGTTQCQR 32	(SEQ ID NO:2)
	MKG C++AWLFSSLG+WRLA PE Q +CQR	(SEQ ID NO:14)
mu semF:	1 MKGACILAWLFSSLGVWRLARPETQDPAKCQR 32	(SEQ ID NO:5)

Underlined - predicted signal peptide

FIG. 4

**Comparison of emxosb4f08 to probable calcium-binding protein (CaBP)
(Genbank Accession number JS0027)**

emxosb4f08	1	<u>MRSLLRTPFLCGLLWAF</u> CAPGARAEEPAASFSQPGSMGLDKN	42
emxosb4f08	43	TVHDQE HIMEHLEGVINKEAEMSPQELQLHYFKMHDYDGNNL	84
		MSPQELQLHYFKMHDYDGNNL	
CaBP	1	MSPQELQLHYFKMHDYDGNNL	21
emxosb4f08	85	LDGLELSTAITHVHKEEGSEQAPL	109 (SEQ ID NO:4)
		LDGLELSTAITHVHKEEGSEQAPL	(SEQ ID NO:15)
CaBP	22	LDGLELSTAITHVHKEEGSEQAPL	45 (SEQ ID NO:6)

Underlined - predicted signal peptide

FIG. 5